

Supplemental Table 1. Associations and correlations of *CTLA4* methylation and mRNA expression with clinicopathological parameters and molecular features in n=470 melanoma patients from The Cancer Genome Atlas. Molecular data were adopted from the Cancer Genome Atlas Network [Genomic Classification of Cutaneous Melanoma. *Cell*. 2015;161(7):1681-96]. P-values refer to Kruskal-Wallis (> two group comparisons), ANOVA with Bonferroni post-hoc pairwise comparisons (table footnote), Wilcoxon Mann-Whitney *U* (2 group comparison) tests, and Spearman's rank correlation (continuous variables), respectively. Significant features are shown in boldface.

Variable	CTLA4 methylation			CTLA4 mRNA expression		
	Total n (%)	Mean methylation [%], [95% CI]	p-value; (Spearman's ρ)	Total n (%)	Mean normalized counts, [95% CI]	p-value; (Spearman's ρ)
All Patients	470	43.1 [41.5-44.7]		468	145 [120-171]	
Age	462		$p=0.058$; $\rho=0.088$	459		$p=0.33$; $\rho=-0.045$
Mean	58.2			58.2		
Median	58.0			58.0		
Range	15 – 90			15 – 90		
Gender	469 (100%)			468 (100%)		
Male	289 (61.6%)	44.0 [41.9-46.1]		289 (61.8%)	145 [116-175]	
Female	180 (38.4%)	41.7 [39.3-44.1]	$p=0.16$	179 (38.2%)	145 [98-192]	$p=0.87$
Tissue	467 (100%)			465 (100%)		
Regional cutaneous or subcutaneous tissue	74 (15.8%)	45.2 [40.9-49.5]		74 (15.9%)	203 [93-313]	
Regional lymph node	222 (47.5%)	40.7 [38.6-42.8]		221 (47.5%)	168 [134-202]	
Primary tumor	103 (22.1%)	46.2 [42.6-49.9]		102 (21.9%)	89 [50-129]	
Distant metastasis	68 (14.6%)	43.3 [38.7-47.9]	$p=0.10^\dagger$	68 (14.6%)	96 [64-127]	$p<0.001^\dagger$
Mutation subtype	316 (100%)			315 (100%)		
<i>BRAF</i> hotspot	150 (47.5%)	39.3 [37.0-41.7]		150 (47.6%)	153 [119-188]	
<i>RAS</i> hotspot	92 (29.1%)	45.7 [41.7-49.8]		92 (29.2%)	162 [66-257]	
<i>NF1</i> mutant	28 (8.9%)	49.4 [43.3-55.6]		27 (8.6%)	156 [57-255]	
Triple wild type	46 (14.6%)	52.3 [47.1-57.5]	$p<0.001^\dagger$	46 (14.6%)	102 [60-144]	$p=0.16$
Pathologic tumor category (pT)	394 (100%)			392 (100%)		
pT ₀	23 (5.8%)	40.1 [33.5-46.6]		23 (5.9%)	177 [69-285]	
pT _{is}	8 (2.0%)	34.5 [18.9-50.1]		8 (2.0%)	96 [21-170]	
pT ₁	42 (10.7%)	36.7 [32.8-40.5]		42 (10.7%)	185 [123-248]	
pT ₂	78 (19.8%)	46.1 [42.5-49.8]		78 (19.9%)	104 [76-133]	
pT ₃	90 (22.8%)	41.5 [37.9-45.1]		89 (22.7%)	209 [116-302]	
pT ₄	153 (38.8%)	47.0 [44.1-50.0]	$p=0.001^\dagger$	152 (38.8%)	91 [61-122]	$p<0.001^\dagger$
Pathologic nodal category (pN)	413 (100%)			412 (100%)		
pN ₀	235 (56.9%)	44.6 [42.3-46.9]		235 (57.0%)	149 [105-192]	
pN ₁	74 (17.9%)	39.0 [35.5-42.6]		74 (18.0%)	152 [99-205]	
pN ₂	49 (11.9%)	46.6 [41.4-51.9]		49 (12.0%)	156 [91-222]	

	pN ₃	55 (13.3%)	41.4 [36.9-45.8]	p=0.054 [‡]	54 (13.1%)	129 [83-175]	p=0.26
Pathologic distant metastases category (pM)		442 (100%)			440 (100%)		
	pM ₀	418 (95.6%)	43.0 [41.3-44.7]		416 (94.5%)	145 [118-173]	
	pM ₁	24 (5.4%)	43.6 [35.9-51.3]	p=0.84	24 (5.5%)	140 [4-276]	p=0.59
Pathologic stage (AJCC 7)		418 (100%)			416 (100%)		
	0	7 (1.7%)	33.8 [15.2-52.4]		7 (1.7%)	104 [19-190]	
	I	77 (18.4%)	42.9 [39.2-46.6]		77 (18.5%)	148 [108-189]	
	II	140 (33.5%)	46.4 [43.4-49.5]		139 (33.4%)	117 [57-178]	
	III	171 (40.9%)	41.5 [39.0-40.7]		170 (40.9%)	152 [120-184]	
	IV	23 (5.5%)	44.7 [37.1-52.4]	p=0.094	23 (5.5%)	143 [0-285]	p<0.001 [‡]
Breslow depth		359		p=0.13; ρ =0.079	359		p<0.001; ρ =-0.210
	Mean	5.58			5.58		
	Median	3.00			3.00		
	Range	0-75			0-75		
Melanoma ulceration indicator					312 (100%)		
	Yes	167	45.0 [42.3-47.8]		166 (53.2%)	109 [79-139]	
	No	146	43.2 [40.4-46.1]	p=0.42	146 (46.8%)	160 [102-217]	p=0.006
	TERT RNA-Seq	328		p=0.15; ρ =-0.080			p=0.82; ρ =0.013
	Pigment score	329		p=0.22; ρ =0.068	328		p=0.82; ρ =-0.013
	Necrosis	329		p=0.15; ρ =-0.080	328		p=0.10; ρ =0.091
Immune cell infiltrates / purity / inflammation							
	Reverse phase protein array (RPPA): LCK	200		p=0.53; ρ =-0.044	199		p<0.001; ρ =0.405
	RPPA: SYK	200		p=0.11; ρ =-0.114	199		p<0.001; ρ =0.395
	Tumor content (% nuclei that are tumor cells)	329		p=0.56; ρ =-0.033	328		p=0.019; ρ =-0.130
	Purity [*]	299		p=0.80; ρ =0.015	298		p<0.001; ρ =-0.479
	Lymphocyte distribution	329		p=0.30; ρ =-0.058	328		p<0.001; ρ =0.278
	Lymphocyte density	329		p=0.19; ρ =-0.073	328		p<0.001; ρ =0.342
	Lymphocyte score	329		p=0.26; ρ =-0.063	328		p<0.001; ρ =0.328
	B cells [†]	468		p=0.056; ρ =-0.088	468		p<0.001; ρ =0.398
	CD4 ⁺ T cells [†]	468		p=0.28; ρ =0.050	468		p<0.001; ρ =0.316
	CD8 ⁺ T cells [†]	468		p=0.007; ρ =-0.126	468		p<0.001; ρ =0.567
	Neutrophils [†]	468		p=0.068; ρ =-0.084	468		p<0.001; ρ =0.517
	Macrophages [†]	468		p=0.25; ρ =0.053	468		p<0.001; ρ =0.248
	Dendritic cells [†]	468		p=0.022; ρ =-0.106	468		p<0.001; ρ =0.614

RNASEQ_CLUSTER_CO	329 (100%)			329 (100%)		
NSENHIER						
MITF-low	59 (17.9%)	36.4 [32.2-40.5]		59 (17.9%)	183 [53-314]	
Keratin	102 (31.0%)	48.4 [44.7-52.1]		102 (31.0%)	87 [47-127]	
Immune	168 (51.1%)	44.2 [41.8-46.6]	p<0.001[‡]	168 (51.1%)	165 [129-202]	p<0.001[‡]
UV signature	318 (100%)			317 (100%)		
Yes	264 (83.0%)	43.3 [41.2-45.4]		263 (83.0%)	157 [118-196]	
No	54 (17.0%)	48.0 [43.5-52.5]	p=0.031	54 (17.0%)	104 [53-155]	p=0.009
Methylation subtype	331 (100%)			330 (100%)		
Normal-like	70 (21.1%)	43.9 [41.0-46.8]		69 (20.9%)	233 [159-308]	
Hypo-methylated	84 (25.4%)	37.8 [34.8-40.8]		84 (25.5%)	105 [72-138]	
Hyper-methylated	93 (28.1%)	43.0 [39.5-46.5]		93 (28.2%)	172 [85-260]	
CpG island-methylated	84 (25.4%)	51.9 [47.3-56.5]	p<0.001[‡]	84 (25.5%)	78 [39-117]	p<0.001[‡]
Methylation						
<i>CDKN2A</i> (cg13601799)	329		p<0.001; $\rho=0.210$	328		$\rho=0.25$; $\rho=-0.064$
<i>KIT</i> (cg10087973)	330		p=0.036; $\rho=0.116$	329		$\rho=0.19$; $\rho=0.073$
Total mutations	319		$\rho=0.61$; $\rho=-0.029$	317		p=0.010; $\rho=0.145$
Mean	849			849		
Median	431			430		
Range	15-49,557			15-49,557		
CC>TT mutations	318		$\rho=0.19$; $\rho=-0.074$	317		$\rho=0.86$; $\rho=0.010$
C>T transitions at dipyrimidines	318		$\rho=0.69$; $\rho=0.023$	317		p=0.001; $\rho=0.189$
C>T transitions at dipyrimidines in relation to total C>T transitions	318		$\rho=0.96$; $\rho=0.003$	317		p=0.004; $\rho=0.163$
Mutation						
<i>ARID2</i>	318 (100%)			317 (100%)		
Wild type	273 (85.8%)	43.0 [41.0-45.0]		272 (85.8%)	162 [123-200]	
Mutant	45 (14.2%)	50.7 [45.1-56.3]	p=0.007	45 (14.2%)	65 [43-86]	$\rho=0.056$
<i>BRAF</i>	318 (100%)			317 (100%)		
Wild type	152 (47.8%)	48.4 [45.5-51.3]		151 (47.6%)	118 [79-156]	
Mutant	166 (52.2%)	40.1 [37.8-42.5]	p<0.001	166 (52.4%)	176 [122-229]	p=0.032
<i>NRAS</i>	318 (100%)			317 (100%)		
Wild type	230 (72.3%)	43.4 [41.2-45.6]		229 (72.2%)	157 [117-197]	
Mutant	88 (27.7%)	45.9 [41.8-49.9]	$\rho=0.36$	88 (27.8%)	125 [64-187]	$\rho=0.18$
<i>KRAS</i>	318 (100%)			317 (100%)		
Wild type	314 (98.7%)	44.0 [42.1-45.9]		313 (98.7%)	149 [115-183]	
Mutant	4 (1.3%)	50.0 [15.3-84.7]	$\rho=0.57$	4 (1.3%)	79 [-8-166]	$\rho=0.90$
<i>HRAS</i>	318 (100%)			317 (100%)		
Wild type	313 (98.4%)	43.9 [42.0-45.8]		312 (98.4%)	138 [113-164]	
Mutant	5 (1.6%)	57.8 [23.1-92.5]	$\rho=0.16$	5 (1.6%)	748 [-1229-2724]	$\rho=0.88$

NF1	Wild type	318 (100%)			317 (100%)		
	Mutant	272 (85.5%)	43.6 [41.6-45.7]		272 (85.8%)	139 [111-167]	
IDH1	Wild type	46 (14.5%)	46.8 [41.3-52.4]	p=0.20	45 (14.2%)	204 [37-372]	p=0.79
	Mutant	318 (100%)			317 (100%)		
GNA11	Wild type	300 (94.3%)	43.2 [41.3-45.1]		299 (94.3%)	152 [117-187]	
	Mutant	18 (5.7%)	58.7 [50.1-67.3]	p<0.001	18 (5.7%)	81 [39-122]	p=0.86
GNAQ	Wild type	318 (100%)			317 (100%)		
	Mutant	311 (97.8%)	43.9 [42.0-45.8]	p=0.25	310 (97.8%)	139 [113-165]	p=0.91
CDK4	Wild type	7 (2.2%)	54.1 [29.5-78.8]		7 (2.2%)	569 [-667-1804]	p=0.91
	Mutant	318 (100%)		p=0.001	317 (100%)		
CDKN2A	Wild type	312 (98.1%)	43.5 [41.7-45.4]		311 (98.1%)	150 [116-184]	
	Mutant	6 (1.9%)	73.0 [54.3-91.8]		6 (1.9%)	62 [-32-157]	p=0.24
DDX3X	Wild type	318 (100%)			317 (100%)		
	Mutant	312 (98.1%)	44.4 [42.2-46.1]	p=0.89	311 (98.1%)	149 [115-183]	p=0.47
KIT	Wild type	6 (1.9%)	41.7 [30.8-52.7]		6 (1.9%)	93 [-50-235]	
	Mutant	318 (100%)		p=0.067	317 (100%)		
MAP2K1	Wild type	276 (86.8%)	44.8 [42.7-46.9]		275 (86.8%)	136 [101-171]	
	Mutant	42 (13.2%)	39.6 [35.5-43.6]		42 (13.2%)	229 [124-334]	p=0.007
PPP6C	Wild type	318 (100%)			317 (100%)		
	Mutant	298 (93.7%)	44.4 [42.4-46.3]	p=0.23	297 (93.7%)	142 [108-176]	p=0.49
PTEN	Wild type	20 (6.3%)	40.0 [32.0-48.0]		20 (6.3%)	239 [63-415]	
	Mutant	318 (100%)		p=0.94	317 (100%)		
RAC1	Wild type	306 (96.2%)	44.2 [42.2-46.1]		305 (96.2%)	150 [115-184]	
	Mutant	12 (3.8%)	42.4 [32.2-52.5]		12 (3.8%)	111 [3-220]	p=0.50
RB1	Wild type	318 (100%)			317 (100%)		
	Mutant	302 (95.0%)	44.0 [42.0-45.9]	p=0.68	301 (95.0%)	148 [113-183]	p=0.60
TP53	Wild type	16 (5.0%)	46.2 [37.3-55.2]		16 (5.0%)	144 [53-235]	
	Mutant	318 (100%)		p=0.87	317 (100%)		
TERT	Wild type	295 (92.8%)	44.1 [42.1-46.0]		294 (92.7%)	150 [115-186]	
	Mutant	23 (7.2%)	44.4 [36.9-52.0]		23 (7.3%)	119 [50-189]	p=78
NF1	Wild type	318 (100%)			317 (100%)		
	Mutant	291 (91.5%)	44.0 [41.9-46.0]	p=0.23	290 (91.5%)	154 [118-190]	
IDH1	Wild type	27 (8.5%)	45.5 [39.8-51.2]		27 (8.5%)	80 [38-123]	p=0.028
	Mutant	318 (100%)		p=0.79	317 (100%)		
GNA11	Wild type	298 (93.7%)	44.1 [42.1-46.0]		297 (93.7%)	144 [110-178]	
	Mutant	20 (6.3%)	44.5 [37.3-51.8]		20 (6.3%)	206 [52-361]	p=0.34
GNAQ	Wild type	318 (100%)			317 (100%)		
	Mutant	306 (96.2%)	44.2 [42.3-46.2]	p=0.74	305 (96.2%)	148 [114-183]	
CDK4	Wild type	12 (3.8%)	40.9 [29.4-52.4]		12 (3.8%)	146 [44-249]	p=0.16
	Mutant	318 (100%)		p=0.86	317 (100%)		
CDKN2A	Wild type	269 (84.6%)	44.3 [42.2-46.4]		268 (84.5%)	146 [108-183]	
	Mutant	49 (15.4%)	43.1 [38.6-47.6]		49 (15.5%)	161 [90-231]	p=0.053
DDX3X	Wild type	113 (100%)			113 (100%)		
	Mutant	39 (34.5%)	45.3 [39.9-50.6]	p=0.80	39 (34.5%)	103 [60-146]	
KIT	Wild type	74 (65.5%)	44.5 [40.4-48.6]		74 (65.5%)	139 [82-197]	p=0.82
	Mutant						

† according to Li *et al.* [Comprehensive analyses of tumor immunity: implications for cancer immunotherapy. *Genome Biol.* 2016;17(1):174]

* according to Carter *et al.* [Absolute quantification of somatic DNA alterations in human cancer. *Nat Biotechnol.* 2012;30(5):413-21]

‡ ANOVA with Bonferroni post-hoc pairwise comparisons (listed are p-values < 0.050): p=0.047 (methylation; regional lymph node vs. primary tumor); p=0.047 (mRNA; primary tumor vs. regional cutaneous or subcutaneous tissue); p=0.024, p=0.021, p<0.001 (methylation; *BRAF* hotspot vs. *RAS* hotspot, *BRAF* hotspot vs. *NF1* mutant, *BRAF* vs. triple wild type); p=0.057, p=0.008 (methylation; pT₁ vs pT₂, pT₁ vs pT₄); p=0.014 (mRNA; pT₃ vs pT₄); not significant (mRNA, pathologic stage); p<0.001, p=0.007 (methylation; MITF-low vs keratin, MITF-low vs immune); not significant (mRNA; RNASEQ_CLUSTER_CONSENHIER); p=0.020, p<0.001, p= 0.003 (methylation; normal-like vs CpG island-methylated, hypo-methylated vs. CpG island-methylated, hyper-methylated vs CpG island-methylated); p=0.043, p=0.007 (mRNA; normal-like vs. hypo-methylated, normal-like vs CpG island-methylated).

Supplemental Table 2. Clinical and pathological data and their associations/correlations with tumor mCTLA4 levels from 50 melanoma patients treated with immune checkpoint blockage (ICB) immunotherapy (ICB-cohort). Significant features are shown in boldface.

Variable	Total n (%)	CTLA4 methylation Mean methylation [%], [95%CI]	p-value; (Spearman's ρ)
All Patients	50	33.2 [24.7-41.7]	
Age	Mean 69.0 Median 72.0 Range 45 – 90		p=0.23; ρ =0.174
Gender	Male 29 (58.0%) Female 21 (42.0%)	40.8 [28.2-53.4] 22.6 [13.0-32.3]	p=0.004
Tissue	Regional cutaneous or subcutaneous tissue 17 (34.0%) Regional lymph node 14 (28.0%) Primary tumor 10 (20.0%) Distant metastasis 8 (16.0%) Unknown 1 (2.0%)	49.3 [30.0-68.6] 13.6 [7.5-19.8] 27.1 [9.0-45.2] 39.1 [21.2-57.0]	p=0.001
Pathologic tumor category (pT) at initial diagnosis	pT ₀ , pT _{is} , pT ₁ 0 (0.0%) pT ₂ 8 (16.0%) pT ₃ 10 (20.0%) pT ₄ 22 (44.0%) Tx 10 (20.0%)	16.1 [3.8-28.3] 44.3 [25.4-63.2] 33.7 [19.0-48.4]	p=0.072
Pathologic nodal category (pN) at initial diagnosis	pN ₀ 9 (18.0%) pN ₁ 7 (14.0%) pN ₂ 11 (22.0%) pN ₃ 16 (32.0%) pN _x 7 (14.0%)	27.9 [10.2-45.5] 20.2 [5.3-35.1] 30.4 [13.4-47.3] 38.2 [17.8-58.6]	p=0.86
Pathologic distant metastases category (pM) at immunotherapy start	pM ₀ 0 (0.0%) pM _{1a} 10 (20.0%) pM _{1b} 10 (20.0%) pM _{1c} 27 (54.0%) Unknown 3 (6.0%)	24.0 [11.0-37.1] 37.0 [19.7-54.2] 33.2 [20.2-46.1]	p=0.38
Mutation			
BRAF	49 (100%) Wild type 32 (65.3%) Mutant 17 (34.7%)	31.0 [20.4-41.6] 36.2 [19.7-52.6]	p=0.72
NRAS	27 (100%) Wild type 15 (55.6%) Mutant 12 (44.4%)	19.9 [11.6-28.3] 37.0 [18.9-55.0]	p=0.13
KIT	11 (100%) Wild type 7 (63.6%) Mutant 4 (36.4%)	22.9 [6.6-39.2] 43.9 [-56.9-144.7]	p=1.0
Response (irRECIST)	Progressive disease 27 (44.0%) Stable disease 17 (34.0%) Partial response 5 (10.0%) Complete response 1 (2.0%)	44.4 [31.2-57.5] 23.2 [12.9-33.5] 12.3 [-1.4-26.0] 4.9	p=0.009†
Therapy history (prior BRAF inhibitor)	Yes 9 (18.0%)	32.2 [10.5-53.8]	

	No	41 (82.0%)	33.4 [23.8-43.1]	p=0.96
Immunotherapy regimen				
Pembrolizumab monotherapy		27 (54.0%)	33.4 [22.5-44.4]	
Nivolumab monotherapy		4 (8.0%)	68.2 [-19.1-155.4]	
Ipilimumab monotherapy		1 (2.0%)	16.2	
Combined therapy (ipilimumab + nivolumab/pembrolizumab)		12 (24.0%)	26.5 [12.0-41.0]	
Sequential therapy (ipilimumab + nivolumab/pembrolizumab)		6 (12.0%)	24.9 [-1-50.4]	p=0.56

† Partial and complete response groups were analyzed as one combined response group